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Gülay, Arda; Musovic, Sanin; Albrechtsen, Hans-Jørgen; Smets, Barth F.

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## Neutrophilic iron oxidizers adapted to highly oxidic environments

A. Gülay, S. Musovic, H.J. Albrechtsen and B.F. Smets

Department of Environmental Engineering, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

### Abstract:

Rapid sand filtration is an economical way to treat anoxic groundwaters and involves aeration followed by particulate and soluble substrate removal via deep bed filtration. The anoxic source groundwater can contain several potential electron donors ( $\text{CH}_4$ ,  $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{NH}_4^+$  and assimilable organic carbon) while oxygen ( $\text{O}_2$ ) is the electron acceptor provided during the aeration process. Numerous previous studies have described neutrophilic iron oxidizers as a bacterial guild with a special niche preference, especially the transition zone between aerobic and anoxic regions, where abiotic chemical oxidation of iron would be retarded. For that reason, no attempts have been documented to describe the density and diversity of iron oxidizing bacteria (FeOB) in oxic neutrophilic environments. Under low temperatures (5 to 10°C) conditions, as typically found in groundwater, extremely low rates of chemical iron oxidation ( $t_{1/2}$ : 315min.) have been documented. This assumed slow chemical oxidation of  $\text{Fe}^{2+}$  in rapid sand filters may allow certain bacteria to oxidize iron concurrently with the ongoing slow chemical oxidation. Hence, we aimed to investigate the abundance, diversity, and spatial distribution of iron oxidizing bacterial in the highly oxidic environments found in typical rapid sand filters.

The neutrophilic FeOB were enriched by the  $\text{Fe}^{2+}/\text{O}_2$  opposing gradient technique and quantified by MPN methodology. Diversity fingerprints of the enrichment cultures were obtained with a 16S rRNA targeted DGGE technique, and dominant bands were isolated and sequenced for identification of dominant enrichment members. Enrichment were microscopically examined via CSLM in combination with FeOB specific or generic cytochromes to verify enrichments, check cell morphologies and quantify cell densities. Our results indicate that neutrophilic iron oxidizers in highly oxidic environments like drinking water treatment systems can be abundant (5 E+04 to 7 E+05 cells per gram of wet sand material). It was furthermore observed that the diversity of the cultivated dominant iron oxidizers differs substantially from those typically observed in aerobic/anoxic transition zones.